Steady-state dynamics and experience-dependent plasticity of dendritic spines of layer 4/5a pyramidal neurons in somatosensory cortex

Amaya Miquelajáuregui1, Sahana Kribakaran1, Ricardo Mostany3, Aurora Badaloni4, Giacomo Consalez4 and Carlos Portera-Cailliau1,2

Depts. of 1Neurology and 2Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA. 3Dept. of Pharmacology, Tulane University School of Medicine, New Orleans, LA. 4Div. of Neurosci., San Raffaele Scientific Inst., Milan, Italy. http://www.porteralab.neurology.ucla.edu

1. Layer 4/5a neurons are labeled in Ebf2:Cre transgenic mice

2. Ebf2+ L4/5a pyramidal neurons are classified in two subsets based on the morphology of their apical tufts.

3. Dynamics and experience-dependent plasticity of apical dendritic spines in Ebf2+ pyramidal L4/5a neurons of barrel cortex

4. Electrophysiological properties and optogenetics (ChR2) in L4/5a neurons

Conclusions
1. Ebf2-Cre is expressed in L4 and L5a. Two subsets of pyramidal L4/5a neurons (simple and complex) are distinguished based on various morphological parameters.
2. Under steady-state conditions in adults, the morphology of the apical tufts and the mean spine density were stable at 0.39 ± 0.05 spines/µm (comparable to L5b).
3. Spine elimination increases 4-8d after sensory deprivation, probably due to input loss.
4. Optogenetic stimulation of L4/5a neurons stimulates L2/3 neurons synthetically.

Introduction
- Cortical layers process sensory information hierarchically from L4 to L2/3 to L5/6. This canonical model has been recently challenged by the observation that subsets of L5 neurons are direct recipients of sensory input from the thalamus.
- Experience-dependent plasticity of dendritic spines has been shown to take place in the adult barrel cortex in L2/3 and L5B neurons, but the effects and timeframes are still inconsistent.
- Here we characterize a subset of L4/5a pyramidal neurons and study the effects of sensory deprivation in the adult barrel cortex using longitudinal in vivo two-photon imaging of dendritic spines.

Methods
1. Adult (3-6 m.o.) Ebf2-iCre transgenic mice (GENSAT).
2. In utero injection of viral vectors (rAAV-2/1-FLEX) at E15.5.
3. Cranial window surgery over somatosensory cortex.
4. Intrinsin Optical Signal (IOS) imaging of barrel cortex (map generated by averaging 30 trials of activity during baseline and contralateral whisker stimulation).
5. Chronic 2-photon imaging of Ebf2-Cre; rAAV-flex-EGFP L4/5a pyramidal neuron apical dendrites every 4 days for 25 days.
7. Sensory deprivation: daily unilateral whisker trimming (contralateral side).
8. Whole-cell patch clamp and ChR2-optogenetic stimulation in slices.

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